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Effect of External Factors on Virus of Seasonal Encephalitis

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The study of the effect of physico-chemical factors on ultravirus gives us the possibility, from one angle, to find a reliable antiseptic means, from the other, characterizes the properties of the virus, important in the problem of antivirus immunity.

Since 1935 we have studied the stability of several viruses to internal influences and reported on the effect of temperature, bile, on the photodynamical action of methylene blue and on the oligodynamical action of silver on virus of typhus fever, herpesencephalitis and infectious ectromelia of mice.

In these tests are clarified the vital fact, for characteristic biological properties of viruses, that during the photodynamic influence of methylene blue, virus of herpesencephalitis and infectious ectromelia of mice are inactivated, but retain their immunogenic properties. Now we turn to the question of the influence of external factors on virus, having in mind a study of the influence on spring-summer and Japanese encephalitis.

There are little data in literature on the conservability of encephalitic virus under the influence of external interactions. Lennet and Smith show that the virus of encephalitis Saint Louis, during freezing, lost its virulence gradually, and after 5 months its titer declined from 10-6 to 10-2. Along with this, in a frozen or dried state, the virus remained virulent even during testing 17 months later. Hirano and Koyama explained, that the optimal concentration of hydrogen ions for virus of Japanese encephalitis is 7.4. The authors consider that the virus is resistant to diastase; to

trypsin lipase, its resistance is notably weaker. Kazahara and co-workers report on the influence of ultrasonic waves on virus of Japanese encephalitis. It was proven that a 10 minute contact in vitro completely killed the virus.

Taking into consideration the possibility of cases of laboratory infections with virus of encephalitis, we considered it especially important to establish the influence of internal interactions on virus of spring-summer and Japanese encephalitis in an uncleaned from the yolk medium state. Workers in laboratories work with this type of material most of all.

We used a 10% emulsion of brain of sick mice for the material to be tested. For verification, after the conformable action, emulsion of the brain was introduced intracerebrally into fresh mice. During storage of the brain emulsion, prepared on a physiological solution, in an icebox at 5°, the pathogenicity of the spring-summer and Japanese encephalitis decreased after 45 days and was lost completely after 60 days.

A 10% virus emulsion, prepared on distilled water with the addition of 1% of normal horse serum, retained its pathogenicity even after 60 and 70 days.

Virus emulsion, prepared on a physiological solution with a 50% content of glycerine, retained its characteristics no less than 2 months. Certain very virulent strains of virus of spring-summer and Japanese encephalitis retained their virulence a year during storage at +5° in conditions where a piece of brain was added to 50% glycerine, although they lost considerable virulence.

Irregardless of the high stability of the virus during storage in cold temperatures and especially in glycerine, still for the maintenance of sufficiently virulent strains it is necessary to pass the virus on fresh mice at least once in a month.

Taking into account the possibility of storing virus in a dried state, we, together with Dolinov, conducted tests on drying various ultraviruses. For this there was prepared a thick virus emulsion, which was diluted in half with a physiological solution with the addition of 10% gum-acacia and was subjected to drying in a modified Flosdorf and Medd apparatus (modified by Dolinov).

Virus dried in such a way retained its original virulence, without it decreasing, for no less than 11 months (lengthier periods to this time have not been tested). Identical results were obtained with virus of spring-summer and Japanese encephalitis and also with viruses of Saint Louis encephalitis, encephalomyelitis of horses and epidemical grippe.

The dried viruses of spring-summer and Japanese encephalitis, tested by intracerebral injections into white mice, 11 months after being put in storage, caused an infection on the 4-5 day with the development of paralysis. Titering of the viruses indicated that they still retained their original titers - the first 10-7 and 10-6 for the second.

From the above data, it is seen that the virus of spring-summer and Japanese encephalitis are stable to the action of low temperatures and retain their virulence duratively in a dried status. The latter condition allows for the storage of the virus in laboratory conditions for a long time without having to subject it to passages.

In the next tests, we tested the action of high temperatures on virus of spring-summer and Japanese encephalitis. For this, a 10% emulsion of brain, prepared on a physiological solution, was held at 14-16° in a thermostat at 37 and at 60 to 70. The virulence was periodically checked by the

injections of it to mice. It was proven that the viruses of encephalitis are unstable to high temperatures; they weaken after 10 days at room temperature (3-4 mice of 10 become ill) and die completely after 20 days.

At thermostatic temperatures the viruses of seasonal encephalitis died after 2 days.

In the water bath the virus of spring-summer encephalitis dies after 10 minutes at 60 and 5 minutes at 70. Virus of Japanese encephalitis dies after 10 minutes at 70 and boiling of either virus brought death after 2 minutes.

The vitality of the viruses was tested in regard to action on it by spirits, ether, acetone and lysol. The viruses of spring and summer and Japanese encephalitis, in 10% brain emulsions, were mixed with equal volumes of the substance being tested. After various periods the material was injected intracerebrally into white mice.

The tests disclosed that, the virus coming in contact with spirits or ether died only after 72 hours, while injected on the second day it still caused infection in half of the animals infected with it. In contact with acetone the virus also died after 72 hours. The fastest action was obtained through the use of lysol; solutions of 1% inactivated the virus after 5 minutes, 3% - 2 minutes, 5% - 1 minute. Thus, 3-5% lysol is a very reliable disinfectant against spring-summer and Japanese encephalitis virus.

For establishment of the photodynamic action of the methylene blue on the viruses of encephalitis, we prepared a solution of blue (Merk 1:25,000) adding it to an equal volume of various growths of virus emulsions (1:100, 1:1000 and 1:10,000) and lighted the mixture with an ordinary electric lamp with a 100 watt bulb at a distance of 15 cm. The exposed mixture was injected

intracerebrally into mice. Controls were unexposed mixtures of these same cultivations of virus with methylene blue and emulsions of virus without methylene blue.

The tests showed multiple and unchanging indications of the inactivation of the spring-summer and Japanese encephalitis virus after 15 minutes contact with the methylene blue during exposure. Methylene blue without exposure and exposure alone showed no effect on the virus.

Such results were obtained only with methylene blue of good quality. The source of light also has sufficient significance. We tried sunlight and light from quartz and were convinced of the ordinary lamp light.

The quartz light in itself shows some effect on the virus. Our tests with it gave unequal results and raised the temperature. This temperature also was unequal and affected the results. The quartz lamp was fixed up with a water cooler which helped control the temperature rise. The emulsion was placed in quartz vessels and thus gave an average thickness of emulsion contact of 0.1 cm. Upon intracerebral injection of this emulsion into mice, indications were that the emulsion was fully inactivated after 2 minutes exposure and a dilution of 1:100.

These tests prove to be good in the preparation of photodynamic and ultraviolet vaccines, which will be reported in later studies.

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Summary

Article 2 - Influence of external factors on virus of seasonal encephalitis, by A. K. Shubladze and M. M. Ananina

Virus of spring-summer and Japanese encephalitis in a 10% emulsion of brain of ill mice, prepared on a physiological solution, are conserved at 5° for 45 days. A virus emulsion, prepared on distilled water with the addition of 1% normal equine serum or 50% glycerine, is conserved at 5° for 70 days. A more permanent storage solution is 50% glycerine with the addition of pieces of brain. Special, virulent virus are conserved at 4-5° for 1 year.

Dry virus has been stored more than 11 months without loss of virulence.

Viruses of seasonal encephalitis are not very stable to warmth. Death results after 20 days at 14-16°, after 48 hours at 37, and after 10-15 minutes at 60-70, and after 1 minute if boiled.

Spirits, ether and acetone kill the virus after 72 hours, 1% lysol after 5 minutes, and 3% lysol after 2 minutes. Lysol is highly recommended for a disinfectant while working with virus of encephalitis.

Inactivation of virus of spring-summer and Japanese encephalitis is noted after 15 minutes of photodynamic action of methylene blueing if the virus emulsion is taken in mixtures of 1:100 and above. Ultraviolet irradiation inactivates virus after 2 minutes.